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Lysis of heart endothelial cells from donor origin by cardiac graft infiltrating cells

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Abstract. Endothelial cells may be involved in the acute rejection of allografts. In the present study, graft infiltrating lymphoid cell lines were propagated from a heart graft at the time of histological diagnosis of rejection. The cell lines containing $CD8^+$ cells lysed donor-derived BLCL and endothelial cells (EC) but not third party BLCL or random EC, suggesting that HLA antigens were recognized. The cell lines containing $CD4^+$ cells only did not lyse any target cells. The lysis of EC without preincubation with gamma interferon (gIFN) indicated that the HLA antigens recognized were class I antigens. These results suggested that lysis of donor EC may be one of the mechanisms involved in rejection.

Key words: Endothelial cells – Graft infiltrating cells – Allograft – Acute rejection – Cell mediated lysis – HLA antigens

Apart from HLA antigens, human endothelial cells (EC) bear antigens specific for the EC-monocyte system [10]. It has been suggested that antigens unique for EC, which are not assessed by screening using monocytes, can give rise to antibodies that cause renal [2] or cardiac [1, 3] allograft rejection. In addition, it has been shown for canine EC [5, 6] and for human fetal EC [4, 11] that these cells can be targets for cell mediated lysis in vitro. Both HLA antigens and EC specific antigens may play a role in the mechanism of EC destruction.

In previous studies we have shown that heart graft infiltrating cells (GIC) can specifically lyse cells of donor origin [9]. In these experiments we used T cell lymphoblasts or EBV transformed B cell lines (BLCL) carrying donor antigens as targets. However, in vivo, the cells that are initially encountered by the immune competent cells from the recipient blood are donor EC. Therefore, the reactivity against EC and in particular donor-EC-specific reactions might be relevant for the study of the process of rejection.

We succeeded n isolating EC from a redundant piece of donor heart. We used these cells to investigate their role in cellular rejection. In the present study we described the lytic capacity of cells infiltrating the donor heart at the time of diagnosis of rejection against EC from this heart. The reactivity was compared with the lysis of other target cells by these GIC.

Materials and methods

Patient. A male patient (HLA type: A2, A11, B15 (62), B35, DR4) received a heart graft (HLA type: A1, A19 (29), B8, B12 (44), DR3, DRw52) at 46 years of age. After prophylactic immunosuppression with OKT3 for 6 days his maintenance therapy was cyclosporin A and low dose steroids. At 36 days posttransplant an endomyocardial biopsy (EMB) showed myocytolysis and the patient was treated for rejection. A concurrently taken EMB was cultured in RPMI 1640 Dutch Modification containing 10% human serum, 10% lymphocult (Biotest) and 0.5% phytohaemagglutinin in the presence of a feeder cell mixture of 10^4 random peripheral blood cells and 10^3 third party BLCL in round bottom 96 well plates. Cells growing from the EMB for 6–7 and 8–9 days after the start of the culture were harvested and plated in 96 well plates. After 49 days of culture the wells were screened for phenotype and cytotoxicity and 8 wells were propagated further and tested for cytotoxicity against EC.

Isolation of EC. EC were isolated from a piece of donor heart trimmed during surgery, and from a random piece of blood vessel left after bypass surgery, according to the method described by Klein-Soyer and Cazenave [8]. In short, the vessel was opened lengthwise with a scissors. The EC sides of the tissues were rinsed with phosphate buffered saline containing calcium and magnesium and were scraped with a scalpel blade. The scalpel was rinsed in culture medium (RPMI 1640:M199 1:1 containing 12.5 mM Hepes and 30% inactivated human serum). The cell suspension was plated in Primaria dishes (Falcon). The medium was changed twice a week and 100 U/ml recombinant gamma interferon (gIFN) (Genzyme, Boston, Mass.) was added 3 days before use of the EC, unless stated otherwise.

The phenotype of the GIC, BLCL or EC was determined according to standard methods [9] on the FACScan using conjugated monoclonal antibodies leu 4 (CD3), leu 2 (CD8), and leu 3 (CD4) (Becton Dickinson) for the GIC and B1.1G6 (anti $\beta 2$ microglobulin)

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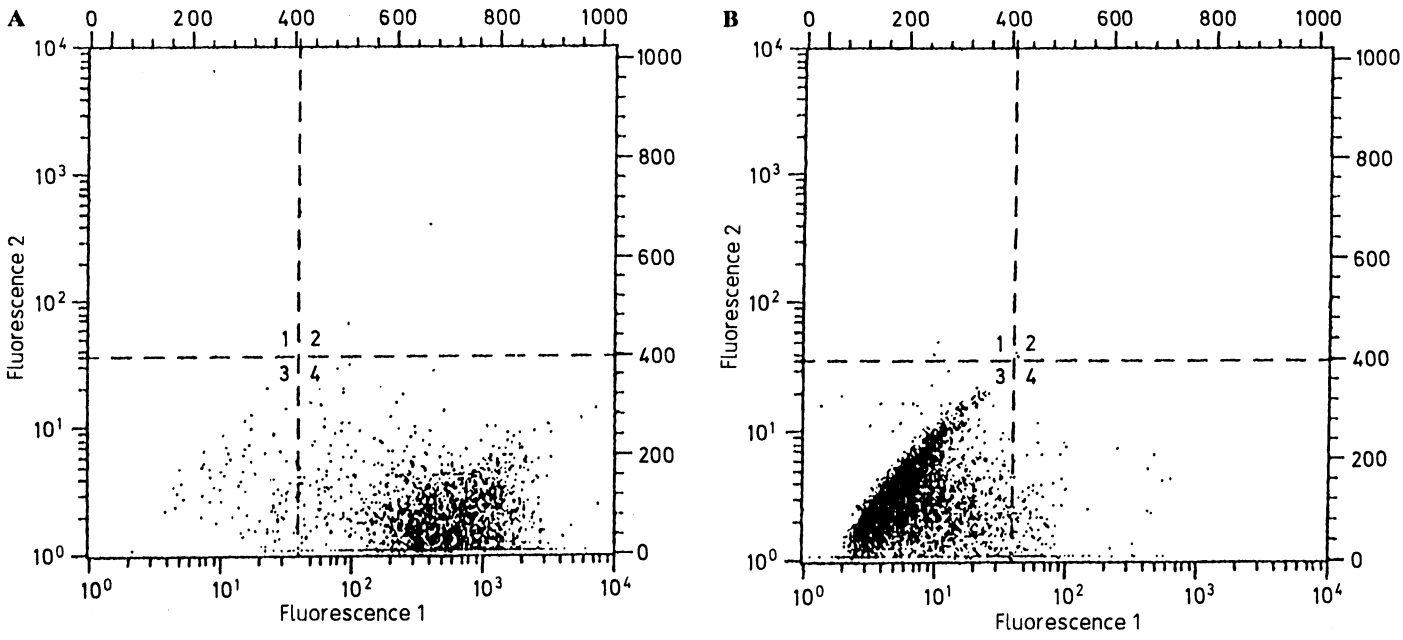

Fig. 1. **A** Cultured donor heart derived EC or **B** random fibroblasts stained with FITC conjugated Ulex Europaeus lectin after trypsinization

plus goat-anti-mouse FITC and anti-HLA DR-FITC for HLA class I and class II antigen measurements respectively. FITC-labeled Ulex Europaeus lectin was used as a marker for EC [7] (Fig. 1). FITC labeled factor VIII was used to stain EC on cytospots.

The cytotoxicity of the GIC was measured by a 4 h ^{51}Cr release test according to standard methods. Target EC were detached by trypsin EDTA (0.05%/0.02%) in PBS without calcium and magnesium and used in the cell suspension.

Results and discussion

After culture in plain medium, EC carried HLA class I antigens but not HLA class II antigens. The addition of gIFN to the cultures induced the expression of HLA class II antigens and slightly increased the expression of HLA class I antigens as measured by FACScan analysis (Table 1). This increase was time dependent and reached maximal values not later than 3 days after the addition of 50 U gIFN/ml. After culture in the presence of gIFN the intensity of expression of HLA antigens on EC was comparable to that on BLCL (Table 1).

T cell lines derived from the heart at the time of diagnosis of rejection and consisting of more than 90% $CD8^+$ cells, lysed both donor BLCL and donor EC (Table 2). Third party BLCL and a random EC line were not lysed. This differential pattern of lysis, in addition to the observation that in a previous test these cell lines did not lyse K562, suggested that HLA antigens present both on BLCL and EC were recognized. The lysis of EC cultured without gIFN, which were shown not to express HLA class II antigens, indicated that the HLA antigens recognized are class I antigens. $CD4^+$ cell lines did not lyse any target cells. The specificity of the reaction will have to be confirmed by blocking studies. We must not exclude the possibility that EC specific antigens as well as HLA antigens are recognized by GIC. We obtained comparable results for three EMB from two other heart transplant recipients i. e. cells cultured from the EMB could lyse both donor derived B cells and donor derived heart EC (results not shown).

It has been shown previously that EC can be targets for cell mediated lysis by peripheral blood cells after an activation phase in vitro [4–6]. The present study showed that

Table 1. Expression of HLA class I and class II antigens on EC in comparison with BLCL

	Class I		Class II	
	positive cells (%)	median fluorescence	positive cells (%)	median fluorescence
BLCL				
Donor	91	586	91	661
3rd party	98	555	98	712
EC				
Donor – IFN	80	445	0	0
Donor + IFN[a]	90	622	72	500
Random – IFN	80	492	0	0
Random + IFN[a]	87	636	82	549

[a] 100 U/ml gIFN was present for 3 days of culture

Table 2. Cell mediated lysis of donor derived BLCL and EC by GIC propagated from an EMB taken at the time of diagnosis of acute rejection

	Specific lysis (%)[a]							
Phenotype	> 90% $CD8^+$ cells					mix	100% $CD4^+$	
Cell line	1	2	3	4	5	6	7	8
BLCL								
Donor	100	96	76	88	58	87	1	0
3rd party	0	2	5	3	1	2	0	2
EC								
Donor – gIFN	47	42	7[c]	30	21	43	9	2
Donor + gIFN[b]	61	52	34	73	68	100	0	5
Random – gIFN	4	9	6	0	5	0	0	2
Random + gIFN[b]	4	2	2	4	0	1	3	3

[a] Effector target ratio 20:1

[b] 100 U/ml gIFN was present for 3 days of culture

[c] Ratio 40:1 19% specific lysis

allograft infiltrating cells activated in vivo can lyse the EC from the specific allograft donor, indicating a role for lysis of EC in the process of rejection.

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